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Morphologic studies on experimental epidemic encephalitis (summer encephalitis) in monkeys (First report). Anatomic and histologic changes, especially of the internal organs, in monkeys infected through the nose

by

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Chiefs: Prof. T. Ogata and Prof. T. Mitamura.

The objective of the investigations which will be reported below is first of all to follow the continuous alterations of the central nervous system and the viscera in experimental epidemic encephalitis (summer encephalitis) in monkeys, and secondly to compare these with those of humans. We also wish to throw some light on the relationship between the spread of the alterations and the method of infection. Since the experiments are not yet terminated, we will limit ourselves to findings especially in the viscera of monkeys infected through the nose.

Method & clinical picture

Ten macaco (Macacus rhesus, body weight 1500-2500 g)

from South Asia were used as experimental animal while as virus material a 10% emulsion of infected mouse brains (Calinina strain) was used. For administration of the virus material we first used the intranasal method. To ensure success; we dripped a very large quantity (ca. 35 cc) into the nasal cavity with or without anesthesia.

Seven animals were infected only once, and at various intervals after the infection the animals were killed with chloroform (animal no. 1 after 2 days, no. 2 after 4 days, no. 6 after 5 days, no. 3 after 6 days, no. 4 after 8 days, no. 12 after 9 days and no. 11 after 11 days.

Animal no. 6: Fever suddenly developed 5 days after the infection and the monkey was killed on the same day.

Animal no. 12: Fever developed after a 6 day period of incubation. Soon thereafter the animal showed signs of agitation and 9 days after the infection paralysis developed. The animal was killed on the same day, i.e. at the stage of paralysis with high fever.

Animal no. 11: Fever developed on the 5th day of sickness, followed 2 days later by agitation. Paralysis was added on the 11th day. On the 12th day of sickness, at the stage of paralysis with a drop in temperature, the animal was killed.

The remaining 3 animals were infected several times.

Animal no. 8: This animal was infected 3 times each

at 5 and 3 day intervals Five days after the last infection the temperature rose. This was followed by agitation and 8 days later by paralysis. The animal died 10 days after the last infection.

Animal no. 9: This animal was infected 3 times each at intervals of 9 and 6 days. After a 5 day period of incubation the temperature rose, followed shortly thereafter by agitation. Paralysis appeared starting from the 8th day. This animal died 10 days after the last inoculation.

Animal no. 10: Infected intranasally again 3 days after the first infection. Starting from the day of the second infection the animal developed severe edema of the face. This monkey died 7 days after the last inoculation without distinct clinical symptoms.

One intact monkey and one treated intranasally with a 10% emulsion of healthy mouse brains served as controls.

Macrosopic and microscopic findings

1. Central nervous system

Aside from the edema and hyperemia of the pia mater and the brain substance which were particularly distinct among the diseased animals there were no unusual macroscopic findings in the brain and spinal cord.

Microscopically we observed the same alterations already described by other authors in human summer encephalitis,

that is to say inflammatory cell infiltration of the pia mater and the brain substance and perivascular cell infiltration, diffuse and circumscribed glial proliferation, hyperemia, capillary bleeding, various degenerative alterations of the cells of the ganglia, necrosis or pallor of the tissues, etc. in the brain and spinal cord. The table gives the gross histologic distribution and intensity of the symptoms.

Table 1. Key: 1. Animal. 2. Fever, agitation. 3. Drop of fever. 4. Paralysis. 5. Found dead. 6. The same. 7. Edema of the face. 8. Frontal lobes. 9. Parietal lobes and temporal. 10. Occipital lobes. 11. Ammon's horn. 12. Cervical spinal cord. 13. Dorsal spinal cord. 14. Lumbar spinal cord. 15. No. in () shows the time intervals after infection.

As can be seen from the table, the animals in which we found no clinical symptoms still revealed slight encephalitic alterations. In the animals (no. 1 and 2) killed shortly after infection the symptoms were confined to the olfactory bulb and the oral segment of the cerebrum, while in the animals killed later (no. 3 and 4) or in one that died without distinct symptoms (no. 10) the symptoms spread further in a caudal direction as far as the spinal cord.

The animal killed on the day of its temperature elevation showed fairly pronounced alterations, the main site of which was the cerebrum. The most striking and most widespread alterations were observed in the animals killed or having died

during the stage of paralysis. It appeared to us that in monkey encephalitis the alterations in the spinal cord are more marked than in human encephalitis.

2. Heart

Macroscopically normal.

Microscopically, in addition to the changes such as capillary bleeding, round cell infiltrations with or without admixture of leukocytes, degenerative alterations in muscle fibers, etc. in the myocardium described by some authors in human encephalitis, there were fairly distinct alterations in the small arteries of the myocardium and subepicardial tissue. These revealed complete disappearance of the finer outlines of the vascular wall. The latter was interspersed with trabecular, coarse-branched or more compactly connected masses. In hematoxylin eosin preparations they were a brilliant red color, in Mallory preparations a fresh rose red and with Weigert fibrin staining they were sometimes blue. Occasionally there were red blood cells in the vascular walls altered in this manner (wall hemorrhages). The elastic fibers had also broken down and disappeared. The endothelial cells did not reveal such altered cells. The vascular lumen was sometimes narrowed. The fiber structure appeared looser at the points interspersed with fibrinoid substance (silver impregnation). We found so-called fibrinoid necrosis or impregnation with plasma or fibrin masses and hemorrhages in the entire layer

of the vascular wall. This condition sometimes extended to the adventitia and its surroundings. Mobilization of histiocytic cells and infiltration predominantly with lymphocytes was also observed. In the surrounding myocardial tissue there was fairly severe edema, infiltration mostly with histiocytes and lymphocytes and lumpy degeneration of the muscle fibers (Fig. 1, 2). With the passage of time, processes of organization set in, resulting in small myocardial callosities (Fig. 3).

The frequency of the above alterations in our experimental animals is shown in Table 2.

Table 2. Key: 1. Animal no. 2. Arterial alteration. 3. Number. 4. Site. 5. Capillary bleeding. 6. Round cell infiltration. 7. Degeneration of muscle fibers. 8. Ventricular septum. 9. Ventricular wall. 10. Subepicardial tissue around the initial portion of the aorta. 11. (scar).

Table 2 shows that the arterial changes are present in 6 out of 10 animals. The acute inflammatory alterations of the myocardium (hemorrhages, cell infiltration, edema, degeneration of the muscle fibers) are not as distinct among animals killed in the stage of paralysis or that died with typical symptoms of disease as in the other animals.

3. Lungs

Macroscopically normal.

Microscopically there were edema and hemorrhages in the

alveolar lumina and septa, as well as slight round cell infiltration around the pulmonary veins. Another notable feature was so-called interalveolitis and peribronchiolitis, mostly in isolated foci. This took the form of histiocytic, lymphocytic and leukocytic infiltration of the alveolar septa and the peribronchial tissues. The quantitative ratios of these cells vary somewhat, but the leukocytes appear to predominate in the severely infilrated parts. A remarkable aspect is the absence of such cell migrations into the alveolar and bronchiole lumina (Fig. 4). So-called megakaryocyte embolisms were occasionally observed. The frequency and intensity of such alterations is shown in Table 3.

Table 3. Key: 1. Animal no. 2. Edema of the alveolar septa and lumina. 3. Edema of the peribronchial tissue. 4. Hemorrhage of the alveolar lumen. 5. Peribronchitis and peribronchial hemorrhages. 6. Round cell infiltration around the pulmonary veins. 7. Interalveolitis and peribronchitis. 8. So-called megakaryocytic embolisms.

The table shows that the lungs of the animals killed shortly after infection had edema of the alveolar septa and lumina of the peribronchial tissue, while so-called interalveolitis and peribronchiolitis were absent. In contrast to this, among the animals that were killed or died later, there was pronounced interalveolitis and peribronchiolitis, but no edema.

So-called megakaryocyte embolisms increased numerically parallel with the intensity of the interalveolitis and peribronchiolitis. Based on these histologic findings and in relationship with the interalveolitis and peribronchiolitis, we believe that the megakaryocyte embolisms, or at least some of them, consist of histiocytic cells.

4. Spleen

Macroscopically the spleen of animals that were killed or died during the stage of paralysis showed slight enlargement. The cut surface revealed stasis of the blood and slight hyperplasia of the pulpa. The lymph follicles, being atrophied, could not be very distinctly seen. Animals killed shortly after infection, on the other hand, had spleens poor in blood and without distinct enlargement. On the cut surface the lymph follicles were very distinct, while the pulpa was not marked by stasis or hyperplasia.

Microscopically the lymph follicles of the spleen of animals autopsied shortly after the infection showed very distinct reaction centers. The follicles were thus also enlarged. There was exudation of plasma or fibrinoid masses from the follicle capillaries and arterioles (referred to as fresh alterations in Table 4)(Fig. 5). As time goes on, however, such follicle reactions become weaker. Edema, proliferative and degenerative alterations of the reticulum cells and hyperemia of the follicle capillaries were also observed.

The plasma and fibrinoid masses were partly absorbed, and partly underwent hyaline transformation. For this reason

the spleens of diseased animals that were killed or died during the stage of paralysis showed an abundance of so-called hyalinosis of the follicles and arterioles (referred to as earlier alterations in Table 4). On the other hand the red pulp of animals autopsied shortly after the infection showed virtually no reaction. Parallel with the symptoms of disease there were moderate reactions in the pulpa, such as an increase in leukocytes and mobilization of the reticuloendothelial cells with erythrophagia and siderophagia. Occasionally stasis was also observed. The enlargement of the spleen of animals autopsied later can thus be regarded as the result of the stasis and hyperplasia of the pulpa. The findings described above are assembled in Table 4.

Table 4. Key: 1. Animal no. 2. Lymph follicles. 3. Fresh alterations. 4. Earlier alterations. 5. Red pulp. 6. Inflammatory reaction. 7. Hyaline transformation. 8. Hemosiderosis. 9. Increase in blood content.

5. Lymphnodes

Macroscopically normal.

Microscopically they reveal the same picture as the spleen. In animals autopsied shortly after the infection there were fresh reactions of the cortical follicles. In the animals that were killed or died later, especially during the stage of paralysis, on the other hand, there were reactions of the sinuses, in the form of sinus catarrh with erythrophagia

and siderophagia (Table 5).

Table 5. Key: 1. Animal no. 2. Cortical follicles. 3. Fresh alterations. 4. Old alterations. 5. Lymph sinus. 6. Sinus catarrh. 7. Erythrophagia. 8. Siderophagia. 9. Control.

6. Liver

Macroscopically the liver, which showed severe fatty degeneration, revealed a picture of nutmeg liver, but no other anomalies.

Microscopically predominantly lymphocyte infiltrations could be seen in Glisson's capsule and the wall of the liver vein. Here again, and more so in the strongly infiltrated parts, there were a few leukocytes and histiocytes. Fatty degeneration of the liver cells and Kupffer's cells was observed as well as very slight submiliary necrosis of the liver cells with accumulation of leukocytes, swelling, slight mobilization of Kupffer's cells, and also accumulation of lymphocytes and histiocytes with or without siderophagia. Occasionally there was very slight siderosis of Kupffer's cells and degenerative alterations of the liver cells. These are shown in Table 6.

Table 6. Key: 1. Animal no. 2.Cellular infiltration, Glisson's capsule. 3. Cellular infiltration of wall of the liver vein. 4. Accumulation of cells and mobilization of liver lobes. 5. Liver cell necrosis with leukocyte infiltration.

6. Leukocytic and histiocytic cell accumulation. 7. Kupffer's cells. 8. Fatty degeneration. 9. Central zone. 10. Intermediate zone. 11. Peripheral zone. 12. Kupffer's cells.

It is apparent from this table that the liver of animals killed shortly after infection reveals more cellular infiltration, while in animals that were killed or died later there was a distinct reaction of Kupffer's cells and fatty degeneration.

7. Kidneys

Macroscopically normal.

Microscopically there was slight degeneration of the urinary tubule epithelium and reactions of some glomeruli, such as necrosis of the loops, nuclear proliferation, hemorrhage and escape of protein-rich plasma filtrate into Bowman's capsule, swelling and formation of semilunar structures in the kidneys of animals killed later.

8. Salivary glands

No noteworthy alterations were observed either macroscopically or microscopically.

9. Adrenals

The lipid content of the cortex was reduced in the animals autopsied later. In animal no. 9 there was a small necrotic focus in the medulla. The other organs and tissues were free from appreciable alterations.

If we glance over the findings described above in all the experimental animals, we are struck by the fact that the changes in the central nervous system in the animals killed shortly after infection were slight and limited to the oral segment of the brain, while in the viscera there were exudations and infiltration of liquid and cellular blood components. On the other hand in the animals that were killed or died later the changes in the central nervous system spread further in a caudal direction to a very pronounced degree. In the viscera the reaction of the reticuloendothelial system dominates the picture.

The writers conclude that:

Summary

(1) The morphologic changes observed in experimental monkey encephalitis are almost identical to those seen in humans;

(2) After intranasal inoculation of large quantities of virus, the virus readily finds its way into the brain alongside the olfactory nerve. Slight alterations develop in the brain from the time of incubation on. The virus also finds its way into the circulation within the first 48 hours and there gives rise to the alterations described above in a certain sequence; and (3)

2. Not only the central nervous system reacts to the encephalitis virus (summer encephalitis), but also the mesenchymal tissue of all the rest of the body, although the reactions of the latter are not as pronounced.

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We wish to thank Prof. Mitamura, Dr. Kitaoka, Dr. Mori and Dr. Tenjin and other colleagues in the Department of Pathology of the Institute for Infectious Diseases for their constant help.

Legends

1. Animal no. 1. Heart. Interstitial myocarditis and fibrinoid necrosis of an artery.

2. Animal no. 4. Heart, as above.

3. Animal no. 8. Heart. Scar formation after fibrinoid necrosis of the artery.

Fig. 4. Animal no. 4. Spleen. Very distinct reaction center with exudation of fibrinoid mass from the follicle capillaries.

5. Animal no. 11. Lungs. Interalveolitis and peribronchiolitis.

FIGURES NOT INCLUDED

Table 1

Animal	1 (2)	2 (4)	3 (6)	4 (8)	6 (5)	11 (11)	12 (9)	8 (9)	5 (9)	10 (7)
Symptom	-	-	-	-	Fever, agitation	Drop of fever, paralysis	Paralysis	Found dead	Found dead	Edema of the face
Bulbus olfactorius	+	++(+++)	++(+++)	+	+	++(+++)	++	+(++)	+	+
Frontal lobe	+	+	++	+	++	+++	+	+	+	+
Parietal lobes and temporal	+	+	+		++	+++	+	+	+	+
Occipital lobes			+		+	+	+	+		
Ammon's horn			+		++	++	+	+	++	+
Thalamus	+	+	++		+	+++	+++	+++	+++	+
N. candatus	+	+	++	+	+	++	+	+	+	+
N. leutiform	+		+	+	++	++	+	+	+	
Cerebellum						+	+		+(++)	+
Pons			+	+		+++	+	+++	++	++
Medulla oblongata			+	+		++	+++	+++		
Cervical spinal cord			+		+	+++	+++	+++	+++	+
Dorsal spinal cord			+			+++	++	++	+	+
Lumbar spinal cord						+++	++	+++	+++	

No. in () shows the time intervals after infection.

No. in () shows the time intervals after infection.

Table 2

Animal number	Arterial alteration		Capillary bleeding	Round cell infiltration	Degeneration of muscle fibers
	Number	Site			
1	2	Ventricular septum	+	+	+
2	0	Ventricular septum	+	+	+
3	2	Ventircular septum	+	+	+
4	2	Ventricular wall Ventricular septum	+	+	+
6	1		±	-	±
11	0	Subepicardial tissue around the initial portion of the aorta			
12	0	++	+	+	+
8	1	Ventricular septum (scar)	-	-	-
9	0		-	-	-
10	1	Ventricular septum	+	±	-

Table 3

Animal number	Edema of the alveolar septa and lumina	Edema of the peribronchial tissue	Hemorrhage of the alveolar lumen	Peribronchitis and peribronchial hemorrhages	Round cell infiltration around the pulmonary veins	Interalveolitis and peribronchitis	So-called megakaryocytic embolisms
1	+	+	+	-	+	-	+
2	+	+	-	-	+	-	+
3	-	-	+(++)	+	-	+(++)	-
4	-	-	++(+)	+	-	+	-
6	-	-	-	-	-	++	±
11	-	-	+	+	-	+++	+++
12	-	-	±	+	-	+(++)	±
8	-	-	++	-	-	+(++)	++
9	-	-	+	-	-	++	++(+++)
10	-	-	+	+	-	+++	+++

Table 4

Animal number	Lymph follicles		Red pulp			
	Fresh alterations	Earlier alterations	Inflammatory reaction	Hyaline transformation	Hemosiderosis	Increase in blood content
1	++	-	-	-	-	-
2	++	±(+)	-	-	±(+)	-
3	+	+	±(-)	+	-	±(+)
4	++	+(++)	±(-)	+	-	±(+)
6	+	++(+)	+	-	-	±(+)
11	+	[illegible]	+(++)	-	++(+++)	++
12	+	[illegible]	+(++)	-	++	++(+)
8	-	[illegible]+	+(++)	+	++	++
9	-	++	+(++)	+	+	++
10	+	+++	±(-)	-	++(+++)	++(+++)

Table 5

Animal number	Cortical follicles		Lymph sinus		
	Fresh alterations	Old alterations	Sinus catarrh	Erythrophagia	Siderophagia
1	+	-	±	-	-
2	+	-	+	-	±
3	±(-)	±	+	+	±
4	-	±	±	-	-
6	-	+	++	±	+
11	-	++(+++)	++	+	++
12	-	++(+)	++	+	++(+++)
8	-	++(+)	+	+	++
9	-	++	+(++)	++	++
10	-	++(+++)	+	+	++
K.1	-	-	+	+	+
K.2	-	-	++	++	++

K = control.

TABLE 6

Animal no.	Accumulation of cells and mobilization of liver lobes						Fatty degeneration			
	Cellular infiltration, Glisson's capsule	Cellular infiltration of wall of the liver vein	Liver cell necrosis with leukocyte infiltration	Leukocytic and histiocytic cell accumulation	Kupffer's cells	Central zone	Intermediate zone	Peripheral zone	Kupffer's cells
1	(±)+	+	-	(±)±	+	-	-	-	-
2	±	±	-	+	±	-	-	-	-
3	±	±	-	+	±	-	-	-	-
4	(±)+	+	-	+	-	-	-	-	+
6	±	±	+	±	±	±	-	-	+
11	±	+	-	-	±	±	±	±	+
12	±	+	-	-	±	(±)+	(±)+	±	±
8	-	-	-	±	±	+	+	(±)+	-
9	-	±	-	±	±	-	-	-	±
10	-	-	-	-	±	-	-	(±)+	(±)+

FIGURES NOT INCLUDED